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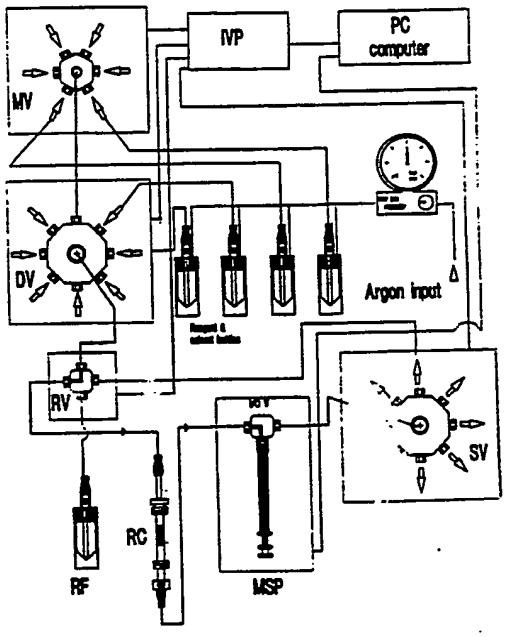
(54) Title: COMPUTER-CONTROLLED, MODULE TYPE, MULTIPURPOSE SYNTHESIZER

(57) Abstract

The present invention provides a computer-controlled, module type, multipurpose synthesizer which contains a liquid flow control means by which the flow of reactants and solvents into the liquid flow control means is controlled by negative pressure and the flow of reactants and solvents out of the liquid flow control means is controlled by positive pressure. This apparatus also contains a directional flow valve means for controlling the direction of flow of reactants and solvents, which allows reactants and solvents to be rapidly passed from the liquid flow control means through the reaction chamber, continuously, rather than being discarded after each pass. This apparatus may also be equipped with a recycling system by which reactants and solvents can be used repeatedly, which can dramatically reduce the amount of expensive monomers usually required. Moreover, the present apparatus may be connected to a fraction collector to measure the progress of synthesis.



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COMPUTER-CONTROLLED, MODULE TYPE, MULTIPURPOSE SYNTHESIZER

The invention described herein was made in the course of work under Grant Nos. CA08748, CA18601 and CA33907 from the National Cancer Institute, National Institutes of Health, U.S. Department of Health and Human Services. Accordingly, the U.S. Government has certain rights in this invention.

Background of the Invention

Throughout this application various publications are referenced within parentheses. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art as known to those skilled therein as of the date of the invention described and claimed in this invention.

The chemical synthesis of organic compounds on solid phase has been vigorously studied during the past 25 years since the first successful demonstration of peptide synthesis by Merrifield (J. Am. Chem. Soc., 85, 2149 (1963)) and oligonucleotide synthesis by Letsinger and Kornet (J. Am. Chem. Soc., 85, 3045). In comparison with the synthesis in solution, the solid support synthesis brought many advantages, especially in the case of oligomers (e.g., oligopeptides and oligonucleotides), because it is possible to omit a large number of purification steps and avoid the isolation of intermediates. At the present, several sophisticated solid phase based synthesizers are commercially available. However, they are generally expensive and their utility is often restricted to the purpose that they were specifically designed for (eg. RNA or DNA synthesis). The commercially available DNA synthesizers use various principles to deliver appropriate reagents and solvents into the reaction vessel (through-flow column). In some cases argon pressure is used and in other cases liquid

transfer is made possible by mechanical pumps (eg. motorized syringe pump, peristaltic pump, continuous piston, or membrane pump) or by combination of both principles (Caruthers et al., U.S. Serial No. 4,458,066, issued July 3, 1984; Melamede, U.S. Serial No. 4,863,849, issued September 5, 1989; Applied Biosystems Model 373A automated DNA sequencing system, Nature, 1990; 347: 310). The mechanical pump is inserted between the liquid reservoirs and the reaction column.

The present invention provides a computer-controlled, module type, multipurpose synthesizer which is readily distinguished from the DNA synthesizers that are commercially available. This synthesizer contains a liquid flow control means, located on the output side of the reaction column, by which the flow of reactants and solvents into the liquid flow control means is controlled by negative pressure and the flow of reactants and solvents out of the liquid flow control means is controlled by positive pressure. Inert gas pressure has only an auxiliary role in balancing the pressure inside the system, so that the possibility of any adverse effect, such as bubble formation or localized high pressure, is eliminated. This apparatus also contains a directional flow valve means for controlling the direction of flow of reactants and solvents, which allows reactants and solvents to be rapidly passed from the liquid flow control means to and through the reaction chamber, continuously, rather than being discarded after each pass. This apparatus may also be equipped with a recycling system by which reactants and solvents can be used repeatedly, which can dramatically reduce the amount of expensive monomers usually required. Moreover, the present apparatus may be connected to a fraction collector or some other suitable instrument, to measure the progress of synthesis. The whole system may also be controlled by a computer. With this invention, it is possible to synthesize efficiently oligomers like oligonucleotides and

oligopeptides in large scale. Moreover, the present invention allows one to synthesize efficiently oligonucleotides and oligopeptides containing modified bases, nucleosides or amino acids which are not naturally occurring. No other system has been developed to meet this demand.

Summary of the Invention

The present invention provides an apparatus for synthesizing an oligomer from preselected reactants which comprises:

(a) multiple reservoirs, each reservoir comprising an input means and an output means, each for holding a different one of the reactants;

(b) multiple reservoirs, each reservoir comprising an input means and an output means, each for holding a different solvent;

(c) a reaction chamber, comprising a first open end and a second open end, for holding a solid support material;

(d) a gas supply means, connected to the input means of the reservoirs, for controlling the supply of gas within the apparatus so as to maintain a constant pressur within the apparatus;

(e) a delivery valve means, comprising an input means and an output means, wherein the input means is connected to the output means of each reservoir and the output means is connected to the first open end of th reaction chamber, for selectively delivering each reactant or solvent from the output means of each reservoir to the first open end of the reaction chamber;

(f) a directional flow valve means, comprising a first port and a second port, wherein the first port is connected to the second open end of the reaction chamber, for controlling the direction of flow of each reactant and solvent;

(g) a liquid flow control means, connected to the directional flow valve means, for controlling the flow of each reactant or solvent into the liquid flow control means by negative pressure and the flow of each excess reactant or solvent out of the liquid flow control means by positive pressure;

(h) a selection valve means, comprising an input means and an output means, wherein the input means is connected to the second port of the directional flow valve means, for controlling the disposition of each excess reactant or solvent flowing from the second port of the directional flow valve means; and

(i) a control means for controlling the selection and delivery of each reactant or solvent by the delivery valve means, the direction of flow of each reactant or solvent by the directional flow valve means, the flow of each reactant or solvent by the liquid flow control means, and the disposition of each excess reactant or solvent by the selection valve means, in response to selected conditions.

The present invention also provides three recycling systems for recycling excess or unused reactants and solvents. In the first system, the apparatus above further comprises a recycling reservoir comprising an input means and an output means, wherein the input means is connected to the output means of the selection valve means and the output means is connected to the input means of the delivery valve means, for holding each excess reactant or solvent before delivery by the delivery valve means.

In the second system, the apparatus above further comprises:

(a) a recycling reservoir, comprising an input/output means, for holding each excess reactant or solvent;

(b) a recycling valve means, comprising a first port, a second port, a third port, and a fourth port, wherein the first port is connected to the output means of the selection valve means, the second port is connected to the input/output means of the recycling reservoir, the third port is connected to the first open end of the reaction chamber, and the fourth port is connected to the output means of the delivery valve means, for recycling each excess reactant or solvent back through the reaction chamber; and

(c) a control means, for controlling the recycling of each excess reactant or solvent by the recycling valve means.

In the third system, the previous system further comprises:

(a) a bidirectional valve means, comprising a first port, a second port, a third port, and a fourth port, wherein the first port is connected to the third port of the recycling valve means, the second port is connected to the first opening end of the reaction chamber, th third port is connected to the second opening end of the reaction chamber, and the fourth port is connect d to the first port of the directional flow valve; and

(b) a control means for controlling the bidirectional valve means.

Additionally, the apparatus of the present invention further comprises:

(a) additional reservoirs, each reservoir comprising an input means and an output means, each for holding an additional reactant;

(b) additional reservoirs, each reservoir comprising an input means and an output means, each for holding an additional solvent;

(c) a modifying valve means, comprising an input means and an output means, wherein the input means is connected to the output means of each additional reservoir and the output means is connected to input means of the delivery valve means, for selectively delivering each additional reactant or solvent to the delivery valve means; and

(d) a control means, for controlling the selection and delivery of each additional reactant or solvent to the delivery valve means by the modifying valve means.

This modifying valve means may be connected to the apparatus in the situation wherein additional reactants and solvents may be required because of the method of synthesis. The modifying valve may be used with or without the above recycling systems.

Lastly, the present invention provides a method for synthesizing an oligomer in a reaction area from preselected reactants which comprises:

(a) displacing one of the preselected reactants by negativ pressure into the reaction area, under suitable conditions for permitting reaction;

(b) removing excess reactant from the reaction area by negative pressure;

(c) repeating steps (a) and (b) a selected number of times until reaction has occurred;

(d) displacing a preselected solvent by negative pressure into the reaction area, under suitable conditions for permitting reaction;

(e) removing solvent from the reaction area by negative pressure;

(f) repeating steps (d) and (e) a suitable number of times until the reaction area is suitably washed;

(g) repeating steps (a)-(c) with the next preselected reactant;

(h) repeating steps (d)-(f) with the next preselected solvent; and

(i) repeating steps (g) and (h) until the oligomer desired has been synthesized.

Brief Description of th Figures

Figure 1. The synthesizer of the present invention with the modifying valve (MV), the delivery valve (DV), the recycling valve (RV), the selection valve (SV), the recycling flask (RF), the reaction column (RC), the motorized syringe pump (MSP), the input/output syringe pump (I/O V), th intelligent valve positioner (IVP), the argon input and inner gas regulator, and reagent and solvent bottles. The recycling valve (RV) and the recycling flask (RF) are not necessary for a working model of the present invention.

Figure 2. Another version of the synthesizer of the present invention with the modifying valve (MV), the delivery valv (DV), the recycling valve (RV), the bidirectional valve (BDV), the reaction column (RC), the motorized syringe pump (MSP), and the selection valve (SV). N1P, N2P, N3P, N4P, and N5P represent various nucleotide positions where reservoirs containing nucleotides are connected, CAP and COP represent the positions where the capping agent and the condensing agent reservoirs are connected, respectively; WIP, WIIP, and WIIIP represent the positions where th washing I, washing II, and washing III solution reservoirs are connected, respectively; DEP, MIP, MIIP, and CTP represent the positions where the deblocking agent, the modifying agent I, the modifying agent II, and the catalyst agent are connected, respectively; WSP and FCP represent the waste position and the fraction collector position, respectively; and RCP represents the recycling position where the recycling reservoir is connected.

Figure 3. Another view of the synthesizer of the present invention showing the PC computer, the intelligent valv positioners (IVPs) (up to 79), the independent automatic valves (up to 316), and the independent automatic syringes (up to 52).

Figure 4. Another version of the synthesizer of the present invention that was used in Examples 1 and 2 with the delivery valve (DV), the reaction column (RC), the motorized syringe pump (MSP), and the selection valve (SV). AA1P, AA2P, AA3P, and AA4P represent the positions where four amino acids are connected; WIP, WIIP, DEP, and RCP represent the positions where the washing I solution, the washing II solution, the deblocking solution, and the recycling reservoir are connected; and BPP and WSP represent the by-pass position and the waste position, respectively.

Figure 5. Another version of the synthesizer of the present invention that was used in Examples 3-6 with the modifying valve (MV), the delivery valve (DV), the reaction column (RC), the motorized syringe pump (MSP), and the selection valve (SV). WIP, WIIP, and WIIIP represent the positions where the washing I, washing II, and washing III solution reservoirs are connected, respectively; DEP, MIP, MIIP, and CTP represent the positions where the deblocking agent, the modifying agent I, the modifying agent II, and the catalyst agent are connected, respectively; N1P, N2P, N3P, N4P, and N5P represent various nucleotide positions where reservoirs containing nucleotides are connected; CAP and COP represent the positions where the capping agent and the condensing agent reservoirs are connected, respectively; and WSP represents the waste position.

Figure 6. HPLC separation of tetrapeptide HOOC-Thr-Leu-Asn-Phe-NH_2 from Example 1.

Figure 7. HPLC separation of decapeptide HOOC-Leu-Phe-Phe-Asn-Leu-Thr-Phe-Thr-Leu-Asn-NH_2 from Example 2.

Figure 8. HPLC Chromatogram of homodecanucleotide d(1Me-Ψ-U)$_{10}$ from Example 3 (see also Table 2). Gradient system; solvent A: 0.1M-TEAA pH 7.00 in water; solvent B: 70%

acetonitrile-water; with flow rate 2.5 ml/min; column type: Dynamax - 300A C18 10x250mm 10-30%B/30 min.

Figure 9. HPLC Chromatogram of homodecanucleotide, decathymidylate $d(T)_{10}$ from Example 4 (see also Table 3). Gradient system; solvent A: 0.1M-TEAA pH 7.00 in water; solvent B: 70% acetonitrile-water; with flow rate 2.5 ml/min; column type: Dynamax - 300A C18 10x250mm 10-30%B/30 min.

Figure 10. HPLC Chromatogram of undecanucleotide $d(T_5$-3Me-Ψ-$U_5)$ (dT = thymidine, d3Me-Ψ-U = 3-methyl-2'-deoxy-Ψ-uridine) from Example 5 (see also Table 4). Gradient system; solvent A: 0.1M-TEAA pH 6.86; solvent B: 70% acetonitrile-water; with flow rate 1 ml/min; column type: Dynamax - C18 10x250mm 5μm 10-50%B/30 min.

Figure 11. HPLC Chromatogram of decanucleotide $d(A\text{-}Me_2\text{-}Ψ\text{-}U)_5$, (dA = 2'-deoxyadenosine, dMe_2-Ψ-U = 2'-deoxy-1,3-dimethyl-Ψ-uridine) from Example 6 (see also Table 5). Gradient system; solvent A: 0.1M-TEAA pH 6.86; solvent B: 70% acetonitrile-water; with flow rate 1 ml/min; column type: Dynamax - C18 10x250mm 5μm 10-25%B/30 min.

Detailed Description of th Invention

The present invention provides an apparatus for synthesizing an oligomer from preselected reactants which comprises:

(a) multiple reservoirs, each reservoir comprising an input means and an output means, each for holding a different one of the reactants;

(b) multiple reservoirs, each reservoir comprising an input means and an output means, each for holding a different solvent;

(c) a reaction chamber, comprising a first open end and a second open end, for holding a solid support material;

(d) a gas supply means, connected to the input means of the reservoirs, for controlling the supply of gas within the apparatus so as to maintain a constant pressure within the apparatus;

(e) a delivery valve means, comprising an input means and an output means, wherein the input means is connected to the output means of each reservoir and the output means is connected to the first open end of the reaction chamber, for selectively delivering each reactant or solvent from the output means of each reservoir to the first open end of the reaction chamber;

(f) a directional flow valve means, comprising a first port and a second port, wherein the first port is connected to the second open end of the reaction chamber, for controlling the direction of flow of each reactant and solvent;

(g) a liquid flow control means, connected to the directional flow valve means, for controlling the flow of each reactant or solvent into the liquid flow control means by negative pressure and the flow of each excess reactant or solvent out of the liquid flow control means by positive pressure;

(h) a selection valve means, comprising an input means and an output means, wherein the input means is connected to the second port of the directional flow valve means, for controlling the disposition of each excess reactant or solvent flowing from the second port of the directional flow valve means; and

(i) a control means for controlling the selection and delivery of each reactant or solvent by the delivery valve means, the direction of flow of each reactant or solvent by the directional flow valve means, the flow of each reactant or solvent by the liquid flow control means, and the disposition of each excess reactant or solvent by the selection valve means, in response to selected conditions.

The present invention also provides three recycling systems for recycling excess or unused reactants and solvents. In the first system, the apparatus above further comprises a recycling reservoir comprising an input means and an output means, wherein the input means is connected to the output means of the selection valve means and the output means is connected to the input means of the delivery valve means, for holding each excess reactant or solvent before delivery by the delivery valve means (see Figure 4).

In the second system, the apparatus above further comprises:

(a) a recycling reservoir, comprising an input/output means, for holding each excess reactant or solvent;

(b) a recycling valve means, comprising a first port, a second port, a third port, and a fourth port, wherein the first port is connected to the output means of the selection valve means, the second port is connected to the input/output means of the recycling reservoir, the third port is connected to the first open end of the reaction chamber, and the fourth port is connected to the output means of the delivery valve means, for recycling excess reactant or solvent back through th reaction chamber; and

(c) a control means, for controlling the recycling of excess reactant or solvent by the recycling valve means (see Figure 1).

In the third system, the previous system further comprises:

(a) a bidirectional valve means, comprising a first port, a second port, a third port, and a fourth port, wherein the first port is connected to the third port of the recycling valve means, the second port is connected to the first opening end of the reaction chamber, the third port is connected to the second opening end of the reaction chamber, and the fourth port is connected to the first port of the directional flow valve; and

(b) a control means for controlling the bidirectional valve means (see Figure 2 and Table 1 for a detailed explanation of this recycling system).

TABLE 1. Explanation of the "Recycling procedure" of the oligonucleotide synthesis according to Figure 2.

Step	Valve position		
	I/O valve	RV	SV
1. Monomer 1 solution suction (DV 0-12)	0	1-4	0-1
2. Monomer solution dispensing from syringe into recycl. flask	0	4-7	0-1
3. Repetition of 1st & 2nd steps as many times as necessary	-	---	---
4. Condensing agent soln. suction (DV 0-2)	0	1-4	0-1
5. Condensing agent soln. dispensing into recycling flask	0	4-7	0-1
6. Repetition of 4th & 5th steps as many times as necessary	-	---	---
7. Preformed mixture suction from recycl. flask through column	I	7-10	0-1
8. Mixture dispensing from syringe into recyclizing flask	0	4-7	0-1
9. Repetition of 7th & 8th steps as many times as necessary	-	---	---
10. Recyclizing flask emptying and collection of unreacted monomer	I 0	7-10 7-10	0-12 0-12
11. Repetition of 10th step as many times as necessary	-	---	---

Additionally, the apparatus of the present invention further comprises:

(a) additional reservoirs, each reservoir comprising an input means and an output means, each for holding an additional reactant;

(b) additional reservoirs, each reservoir comprising an input means and an output means, each for holding an additional solvent;

(c) a modifying valve means, comprising an input means and an output means, wherein the input means is connected to the output means of each additional reservoir and the output means is connected to input means of the delivery valve means, for selectively delivering each additional reactant or solvent to the delivery valve means; and

(d) a control means, for controlling the selection and delivery of each additional reactant or solvent to the delivery valve means by the modifying valve means.

This modifying valve means may be connected to the apparatus in the situation wherein additional reactants and solvents may be required because of the method of synthesis. The modifying valve may be used with or without the above recycling systems.

The apparatus of the present invention may be employed in the synthesis of oligomers such as oligonucleotides and oligopeptides as well as oligonucleotides and oligopeptides containing modified bases, nucleotides or amino acids which are not naturally occurring. Preferably, the invention may be used for the synthesis of oligonucleotides and oligopeptides.

The method of synthesis and the choice of reactants and solvents will depend upon the specific oligomer desired and are readily determined by one skilled in the art. For example, the methods chosen in the examples which follow demonstrate the synthesis of oligopeptides using the FMOC procedure and the synthesis of oligonucleotides using the H-phosphonate method. However, this invention should not be construed to be limited to the methods above. Other methods of synthesis of oligomers may be employed. In addition to the choice of reactants and solvents, the parameters for synthesis such as the flow rate, time, and total volume of reactants and solvents depend on the method of synthesis.

The term "reservoirs" refers to flasks or bottles or other suitable means for containing reactants and solvents. The term "reactants" includes reactants used for carrying out the synthesis desired. For oligonucleotide or oligopeptide synthesis, the reactants may include amino acids or nucleotides. "Reactants" also includes deblocking agents, coupling agents, condensing agents, oxidizing agents, hydrolyzing agents, capping agents, and catalyst agents. The term "solvents" includes all solvents or reagents used for carrying out the synthesis desired. For oligonucleotide or oligopeptide synthesis, the solvents include suitable washing agents.

The synthesis is carried out in the "reaction chamber." The reaction column may be any chamber readily available to those skilled in the art. Preferably, the reaction chamber is a glass column with an adjustable length bed. The "solid support" may be any solid support used by those skilled in the art for oligomer synthesis and depends on the methods chosen for synthesis. Examples of solid support material, although not construed to be limited to the following, include methylbenzhydrylamine resin and long chain alkylamine controlled pore glass.

The "gas supply means" includes any means for controlling the supply of gas within the apparatus so as to maintain a constant pressure within the apparatus. This is used to prevent any adverse effects such as bubble formation or localized high presssure, which may impede the efficiency of synthesis. However, it should be understood that the gas supply means has only an auxiliary role and does not control the flow rate and direction of flow of reactants and solvents. Preferably, the gas is an inert gas such as argon. However, this invention should not be construed to be limited to the inert gas argon and includes other gases known to those skilled in the art that are useful for maintaining a constant pressure during synthesis.

The "delivery valve means" is any delivery valve means for selectively delivering each reactant or solvent from each reservoir to the first open end of the reaction chamber. The delivery valve means may be one valve or a plurality of valves. Such valves are commercially readily available to those skilled in the art and may include but are not limited to Hamilton valves, solenoid valves, or other valves of equal precision. Most preferably, the valves are a daisy chain of Hamilton automatic dispensers.

The "directional flow valve means" is any directional flow valve means for controlling the direction of flow of each reactant or solvent. Such valves are commercially readily available to those skilled in the art and may include but are not limited to Hamilton valves, solenoid valves, or other valves of equal precision. Most preferably, th valves are a daisy chain of Hamilton automatic dispensers. The directional flow valve means controls the direction of flow of reactants or solvents by permiting the reactants or solvents to flow from the reaction chamber into the liquid flow control means and then to the selection valve means or back to the reaction chamber.

Th "liquid flow control means" may be any liquid flow control means which employs negative pressure for controlling the flow of reactants or solvents into itself and positive pressure to control flow of reactants or solvents out of itself. Preferrably, the liquid flow control means is a syringe. Most preferably, the liquid flow control means is a motorized syringe pump.

The "selection valve means" is any selection valve means for controlling the disposition of excess reactants and solvents flowing from the output side of said liquid delivery means. The selection valve means may be one valve or a plurality of valves. Such valves are commercially readily available to those skilled in the art and may include but are not limited to Hamilton valves, solenoid valves, or other valves of equal precision. Most preferably, the valves are a daisy chain of Hamilton automatic dispensers. The selection valv means is further connected to a waste reservoir, a recycling system, or both. The excess reactants and/or solvents may then be disposed as waste through the waste reservoir or recycled via the recycling systems. Additionally, the selection valve means may be connected to a fraction collector or some other suitable instrument, eg. for measuring the progress of synthesis (stepwise yield UV measurement).

The recycling systems allow excess reactants or solvents to be stored and recirculated through the reaction column. In the second and third recycling systems, the "recycling valve means" may be one valve or a plurality of valves. Such valves are commercially readily available to those skilled in the art and may include but are not limited to Hamilton valves, solenoid valves, or other valves of equal precision. Most preferably, the valves are a daisy chain of Hamilton automatic dispensers. In all three systems, the "recycling reservoir" may be one or more flasks or bottles or other suitable means for containing reactants and solvents. The

"bidirectional valve means" in the third system is preferably a bidirectional valve. Such a valve is commercially readily available to those skilled in the art and may include but is not limited to Hamilton valves, solenoid valves, or other valves of equal precision.

Additional valves may be required when the method of synthesis so dictates. This invention permits connecting addititional valves via the "modifying valve means." The modifying valve means gives the user the flexibility of adding a varying number of reactant and solvents that otherwise could not be added using one valve means. Preferably, the modifying valve means may be one valve or a plurality of valves. Such valves are commercially readily available to those skilled in the art and may include but are not limited to Hamilton valves, solenoid valves, or other valves of equal precision. Most preferably, the valves are a daisy chain of Hamilton automatic dispensers. The modifying valve means may be connected to the delivery valve means as shown in Figures 1, 2, and 5.

When using the modifying valve means, additional reservoirs are used for holding the additional reactants and solvents. These reservoirs may be one or more flasks or bottles or other suitable means for containing reactants and solvents.

The various elements of the apparatus, eg. various valve means, liquid delivery means, and reservoirs are connected by tubes that permit the flow of reactants and solvents from one part of the apparatus to another. These connecting tubes are readily commercially available.

The delivery valve means, the directional flow valve means, the liquid flow control means, the selection valve means, the recycling valve means, the bidirectional valve means, and the modifying valve means are all controlled by a control means. The control means is preferably a computer.

Computer programs may be written by one skilled in the art to control the various elements of the apparatus and conditions for synthesis, eg. the flow rate, time, and total volume of reactants and solvents sucked through the reaction chamber.

When the above recited valve means and liquid flow control means are Hamilton valves and syringes, respectively, they are controlled by the computer via Hamilton IVPs (Intelligent valve positioners). However, this invention is not construed to be limited to the use of the Hamilton IVPs and any other system that controls the actions of valves or syringes in response to computer commands may be used. The flexible nature of the present invention permits the use of up to 79 IVPs, 316 independent automatic valves, and 52 independent automatic syringes (see Figure 3).

Lastly, the present invention provides a method for synthesizing an oligomer in a reaction area from preselected reactants which comprises:

(a) displacing one of the preselected reactants by negative pressure into the reaction area, under suitable conditions for permitting reaction;

(b) removing excess reactant from the reaction area by negative pressure;

(c) repeating steps (a) and (b) a selected number of times until reaction has occurred;

(d) displacing a preselected solvent by negative pressure into the reaction area, under suitable conditions for permitting reaction;

(e) removing solvent from the reaction area by negativ pressure;

(f) repeating steps (d) and (e) a suitable number of times until the reaction area is suitably washed;

(g) repeating steps (a)-(c) with the next preselected reactant;

(h) repeating steps (d)-(f) with the next preselected solvent; and

(i) repeating steps (g) and (h) until the oligomer desired has been synthesized.

The term "reactants" includes reactants used for carrying out the synthesis desired. For oligonucleotide or oligopeptide synthesis, the reactants may include amino acids or nucleotides. "Reactants" also includes deblocking agents, coupling agents, condensing agents, oxidizing agents, hydrolyzing agents, capping agents, and catalyst agents. The term "solvents" includes all solvents or reagents used for carrying out the synthesis desired. For oligonucleotide or oligopeptide synthesis, the solvents include suitable washing agents.

The present invention is illustrated in the Experimental Details section which follows. This section is set forth to aid in an understanding of the invention but is not intended to, and should not be construed to, limit in any way the invention as set forth in the claims which follow thereafter.

Experimental Details

EXAMPLE 1

Synthesis of tetrapeptide HOOC-Thr-Leu-Asn-Phe-NH_2

Synthesizer configuration: see Figure 4.

Solid support: 4-Methylbenzhydrylamine Resin, 1% crosslink, 100-200 mesh particle size, substitution 0.35 MEQ/G.

Synthetic method: FMOC, active esters.

Amino acids: Pentafluorophenylester of N-fluorenylmethyl-oxycarbonyl derivatives.

Solvents & reagents: N,N-dimethylformamide (DMF) (WGI), 20% (v/v) piperidine in DMF (DEFMOC), acetonitrile (WGIII).

Reaction column: 8 mm ID glass column with adjustable length of the bed.

Scale: 0.1 mmol (= 287 mg of solid support).

Concentration: 0.05 mol/1 solution of fully protected amino acid in DMF, 2 fold molar excess of each amino acid per working cycle.

The course of the synthesis followed from Figure 4. Th synthetic method consisted of each cycle of deblocking (DEFMOC), washing (WGI), coupling reaction with the use of recycling (COREC), and recycle-type washing (RWGI). The first cycle was preceded by washing (WGI) and the last one was followed by the final washing (WGI, WGIII). The volumes, flow rates, and times for DEFMOC, WGI, COREC, RWGI, and WGIII were chosen according to the FMOC synthetic method.

The amino acid solutions were filled into the appropriate bottles and connected to the AAP positions. Similarly, the dimethylformamide bottle was connected to WIP, piperidine/DMF solution to the DEP, and acetonitrile to the WGIII position. The MSP pump (I-position) then sucked and took WIP of the DV-valve to the column RC; then, MSP was switched to O-position and the solvent was released from the syringe through the waste position WSP of SV to the waste. Then the cycle started with deblocking where the piperidine/DMF solution was taken from the flask connected to the DEP position on DV to the column RC by sucking (I-position) into MSP. The MSP was switched into the O-position, and the solvent was released through the SV valve (WSP-position) into waste. The washing step first started by sucking liquid from the flask in the WIP position on DV. The liquid then went through the reaction column (RC) and entered MSP through the open I-position. The MSP switched to the O-position, and the liquid was released through WSP to waste.

The next step was the coupling operation which was carried out with recycling as follows: The amino acid solution was taken from the appropriate storage flask by suction of MSP (I-position) and passed through the AAP position of DV to the column RC where the coupling reaction took place; it passed on MSP then MSP was switched to the O-position, and the solution passed through by-pass BPP position in SV back to the recycling flask; from there it was taken again by suction, it passed the RCP position on DV and to column RC, then it was driven by MSP to by-pass and returned to recycling flask, etc. up to the amount of predetermined cycles. Washing RWGI then started whereby DMF was sucked through WIP/DV into the column and by-passed in the same way as above into the recycling flask, etc. up to the required time.

The main cycle end d and the next one started with deblocking (DEFMOC) as described above, etc., until all the synthetic cycles were over. After the last one, the WGI and WGIII washings were performed in the mode without recycling.

After synthesis, the dry resin was stirred in a mixture consisting of liquid hydrogen fluoride (18ml), anisole (1ml) and dimethylsulfide (1ml) at 0°C. Hydrogen fluoride and dimethylsulfide were removed by passing nitrogen through the mixture for one hour. The resulting suspension was then triturated with ether. The solid was collected by filtration, and the product was extracted with 60% aqueous acetic acid. The resin was removed by filtration, and the filtrate was lyophilized to give crude peptide (32.5 mg (66% yield). The crude peptide was purified by semipreparative high pressure liquid chromatography on a reverse phase column (μBondapak C18) in water-acetonitrile gradient system contained 0.05% trifluoroacetic acid. After lyophilization of an appropriate fraction, pure tetrapetide HOOC-Thr-Leu-Asn-Phe-NH_2 (21.4 mg 43%) was obtained. Amino acid analysis: Found: Thr, 0.97; Leu, 1.1; Asn (as Asp), 0.97; and Phe, 0.98 (see Figure 6 for HPLC data).

EXAMPLE 2

Synthesis of decapeptide HOOC-Leu-Phe-Phe-Asn-Leu-Thr-Phe-Thr-Leu-Asn-NH_2

Synthesizer configuration: see Figure 4.

Solid support: 4-Methylbenzhydrylamine Resin, 1% crosslink, 100-200 mesh particle size, substitution 0.35 MEQ/G.

Synthetic method: FMOC, active esters.

Amino acids: Pentafluorophenylester of N-fluorenylmethyl-oxycarbonyl derivatives.

Solvents & reagents: N,N-dimethylformamide (DMF) (WGI), 20% (v/v) piperidine in DMF (DEFMOC), acetonitrile (WGIII).

Reaction column: 8 mm ID glass column with adjustable length of the bed.

Scale: 0.05 mmol (= 144 mg of solid support).

Concentration: 0.05 mol/1 solution of fully protected amino acid in DMF, 2 fold molar excess of each amino acid per working cycle.

The procedure followed the same arrangement as in Example 1, except only the numbers of synthetic cycle were different (as for decamer). In this example, only four bottles containing amino acid solution were connected to the DV valve because only Leu, Phe, Thr, and Asn were used in the sequence. More bottles can be connected to the four AAP valves as needed. The same method as in Example 1 was used for coupling with recycling and for the final washing (RWGI).

The work up was the same as in Example 1. The yield of crude peptide was 28.7 mg (43%) of the decamer. Following purification of this product by semipreparative HPLC on reverse phase (μBondapak C18) in water-acetonitrile gradient system contained 0.05% trifluoroacetic acid and lyofilization, 19.0 mg (31%) of pure peptide HOOC-Leu-Phe-Phe-Asn-Leu-Asn-NH2 was isolated. Amino acid analysis: Found: Thr, 2.01; Leu, 2.97; Asn (as Asp), 2.10; and Phe, 2.89 (see Figure 7 for HPLC data).

EXAMPLE 3

Synthesis of homodecanucleotide d(1Me-Ψ-U)$_{10}$

Synthetizer configuration.: see Figure 5.

Solid support: Long chain alkylamine controlled pore glass (CPG) 80-120 mesh particle size, substitution 30.5 micromole of 5'-O-dimethoxytrityl-3'-O-succinyl-1-methyl-2'-deoxy-Ψ-uridine per gram of CPG.

Synthetic method: H-phosphonate method of oligonucleotide synthesis.

Nucleotide: 5'-O-dimethoxytrityl-1-methyl-2'-deoxy-Ψ-uridine-3'-O-(H)-phosphonate.

Condensing agent: Trimethylacetyl chloride (pivaloyl chloride, PVCL).

Solvents & reagents: Acetonitrile (WGIII), 1,2-dichloroethane (WGI), 50% pyridine in acetonitrile (WGII), 2.5% dichloroacetic acid in 1,2-dichloroethane (DBLC), 2% iodine solution in pyridine(98)-water(2) (MDI).

Reaction column: 6.5 mm ID glass column with adjustable length of the bed.

Scale: 0.5 micromole (= 16.4 mg of modified CPG).

Nucleotide conc.: 0.1 mol/l solution of fully protected nucleotide in WGII (15 fold molar excess per working cycle).

Condensing agent conc.: 0.5 mol/l solution of PVCL in WGII (Ration condensing agent:nucleotide = 5:1).

The synthesis proc eded according to the arrangement shown in Figure 5. The synthetic method consisted of the deblocking step (DBLC), 1,2-dichloroethane washing (WGI), pyridine/acetonitrile washing (WGII), coupling step (CO), WGII washing and WGI washing. The first cycle was preceded by WGI washing and the last one followed by DBLC, WGI, and WGIII washings, iodine oxidation (MDIOX), and WGII and WGIII washings.

The reservoirs containing the reagents and solvents were connected to the appropriate positions on the 6-way MV valve and DV valve. The nucleotide monomer solution was connected to the N5P position while N1P-N4P positions, reserved for four basic monomer solutions (dA-N1P; dG-N2P; dC-N3P; dT-N4P), were not used in this case when the title homooligomer was synthesized. The COP position served for coupling reagent, the MIP for oxidizing agent, the DEP position for deblocking solution, and WIP, WIIP, and WIIIP were connected to the appropriate washing solutions. The CAP and CTP positions were vacant in this case. All the steps within the cycle and also the preceding and final steps proceeded in the same simple mode; no recycling system was employed here.

The first step in the cycle, deblocking, started with the sucking preformed by MSP pump (I-position) which drove the deblocking solution from the storage bottle through the DEP position of the MV valve into the DV valve (position 1 in Figure 5) (central outlet) to the column RC. When the MSP piston stroke was completed, the liquid was expelled from the MSP pump (O-position), entered the SV valve and was released through position 4 to the test tube in the fraction collector (the orange colored solutions were collected in each deblocking step and kept for the stepwise-yield UV measurements). After the solution was expelled, the valve on the MSP pump was switched to the I-position again and the syringe started sucking. The WGI washing solvent was driven

through the WIP position to the DV valve (position 1), then to the column RC, and finally to the syringe MSP. The piston of the syringe drove the liquid through the O-position to the SV valve and from there, through the WSP position, to the waste bottle. Now the sucking of the WGII solvent started as described previously. All the solutions, other than the deblocking ones were driven to waste. All the steps throughout the cycles followed this mode, so that only the waste or fraction collector position of SV valve was used.

Each cycle yielded 94.5% (determined spectrophotometrically at 504 nm after each cycle as dimethoxytrityl cation). After synthesis, the CPG-bound oligomer was treated with concentrated ammonium hydroxide solution (min. 29%) for 2 hours at room temperature to release the oligomer from the insoluble solid support. The solid support was removed by centrifugation and the supernatant was evaporated using SpeedVac Concentrator (centrifuge evaporator). The solid residue was additionally heated with concentrated ammonium hydroxide solution (min. 29%) for 6 hours at 55°C. In this example, however, this last step was not necessary. Evaporation of this solution using the same technique (see above) yielded a white foam of the crude oligomer. Additional purification by semipreparative HPLC on the reverse phase column (Dynamax-300A C18 5 microns Rainin Co.) in 0.1 mol/L triethylammonium hydrogencarbonate buffer pH 7.01 solvent system (see below) afforded, after desalting of the oligomer on 10DG column (Bio-Rad Co.), 0.095 μmole (19%) of chromatographically (HPLC) homogeneous decamer (see Table 2 and Figure 8 for results).

TABLE 2.

Data: d(1MePsU)10No7G10-30/30-001

Peak No.	Time	Type	Height(μV)	Area(μV-sec)	Area%
	6.288	N	531	4805	0.117
1	8.121	N	2286	27666	0.676
	9.776	N	1056	4833	0.118
2	10.580	N	6515	64010	1.566
3	11.180	N	2961	16562	0.405
4	11.763	N	6543	77345	1.892
5	12.301	N1	5839	44558	1.090
6	12.453	N2	7840	63241	1.547
7	12.928	N1	9468	92943	2.273
8	13.396	N2	11488	114177	2.793
9	13.796	N3	10509	118167	2.891
10	14.215	N4	12560	161408	3.948
11	14.586	N5	21476	209992	5.137
12	14.883	N6	248900	2127450	52.049
13	15.123	N7	12770	101541	2.484
14	15.548	N8	33874	617828	15.115
15	15.735	N9	19799	220388	5.391
16	17.251	N	741	13729	0.335
	25.323	N	316	5873	0.143
	27.471	N	125	883	0.021
Total Area				4087399	99.991

EXAMPLE 4

Synthesis of homodecanucleotide, decathymidylate $d(T)_{10}$.

Synthetizer configuration: see Figure 5.

Solid support: Long chain alkylamine controlled pore glass (CPG) 80-120 mesh particle size, substitution 27.6 micromole of 5'-O-dimethoxytrityl-3'-O-succinyl-2'-deoxythymidine per gram of CPG.

Synthetic method: H-phosphonate method of oligonucleotide synthesis.

Nucleotide: 5'-O-dimethoxytrityl-2'-deoxythymidine-3'-O-(H)-phosphonate triethylammonium salt.

Condensing agent: Trimethylacetyl chloride (pivaloyl chloride, PVCL).

Solvents & reagents: Acetonitrile (WGIII), 1,2-dichloroethane (WGI), 50% pyridine in acetonitrile (WGII), 2.5% dichloroacetic acid in 1,2-dichloroethane (DBLC), 2% iodine solution in pyridine-water (98.2) (MDI).

Reaction column: 6.5 mm ID glass column with adjustable length of the bed.

Scale (loading): 0.5 micromole (= 18.1 mg of modified CPG).

Nucleotide conc.: 0.1 mol/l solution of fully protected nucleotide in WGII (15 fold molar excess per working cycle).

Condensing agent conc.: 0.5 mol/l solution of PVCL in WGII (Ration condensing agent:nucleotide = 5:1).

The synthesis was performed by alternating delivery of nucleotide and condensing agent in Nx segments into the reaction column (as in Example 3). All steps in the cycles proceeded in the same way as described in Example 3. The thymidine solution was connected to the N4P position while the N1P-N3P and the N5P positions were vacant. Repetitive yield: 97% (determined spectrophotometrically at 504 nm after each cycle as dimethoxytrityl cation). Work up: The same as in Example 3. Yield: 0.28 umole (56%) of chromatographically (HPLC) homogeneous decamer (see Table 3 and Figure 9 for results).

TABLE 3.

Data: d(T)10No12G10-30/30-001

Peak No.	Time	Type	Height(μV)	Area(μV-sec)	Area%
	6.068	N1	513	2171	0.068
	6.193	N2	186	740	0.023
1	9.225	N	1893	15752	0.499
2	13.485	N	2158	18770	0.594
3	14.070	N	2216	20004	0.634
	14.343	N	72	281	0.008
4	16.423	N	2410	20260	0.642
5	17.391	N	2916	24035	0.761
6	17.653	N	2209	16633	0.527
7	18.408	N	3027	40533	1.284
8	18.933	N1	6423	56955	1.805
9	19.346	N2	5206	46114	1.461
10	19.686	N1	8477	80909	2.564
11	19.983	N2	29771	239641	7.595
12	20.216	N3	250833	2137549	67.748
13	20.770	N4	10122	159547	5.056
14	21.038	N5	6162	49288	1.562
15	21.240	N6	8888	158544	5.024
16	22.4[illegible]	N	1807	23897	0.757
	23.018	N	1106	8826	0.279
17	23.421	N	2861	34703	1.099
Total Area				3155152	99.990

EXAMPLE 5

Synthesis of undecanucleotide d(T_5-3Me-Ψ-U_5) (dT = thymidine, d3Me-Ψ-U = 3-methyl-2'-deoxy-Ψ-uridine).

Synthetizer configuration: see Figure 5.

Solid support: Long chain alkylamine controlled pore glass (CPG) 80-120 mesh particle size, substitution 27.6 micromole of 5'-O-dimethoxytrityl-3'-O-succinyl-thymidine per gram of CPG.

Synthetic method: H-phosphonate method of oligonucleotide synthesis.

Nucleotide(s): 5'-O-dimethoxytrityl-thymidine-3'-O-(H)-phosphonate and 5'-O-dimethoxytrityl-2'-deoxy-3-methyl-Ψ-uridine-3'-O-(H)-phosphonate (both compounds as triethylammonium salts).

Condensing agent: Trimethylacetyl chloride (pivaloyl chloride, PVCL).

Solvents & reagents: Acetonitrile (WGIII), 1,2-dichloroethane (WGI), 50% pyridine in acetonitrile (WGII), 2.5% dichloroacetic acid in 1,2-dichloroethane (DBLC), 2% iodine solution in pyridine-water(98.2) (MDI).

Reaction column: 6.5 mm ID glass column with adjustable length of the bed.

Scale (loading): 0.5 micromole (= 18.1 mg of modified CPG).

Nucl. concentration: 0.1 mol/l solution of fully protected nucleotides in WGII (15 fold molar excess per working cycle).

Condensing agent conc.: 0.5 mol/l solution of PVCL in WGII (Ration condensing agent:nucleotide = 5:1).

The synthesis involved alternating delivery of nucleotide and condensing agent in Nx segments into the reaction column (as in Example 3). All of the steps were performed in the same manner as described in Example 3. The N4P position was connected to the thymidine solution bottle while 3-methyl-Ψ-2'-deoxyuridine was connected through the N5P position. Repetitive yield: 95% (determined spectrophotometrically as dimethoxytrityl cation at 504 nm after each cycle). Yield: 0.20 μmole (40%) of chromatographically (HPLC) homogeneous decamer (see Table 4 and Figure 10 for results).

TABLE 4.

Data: d(T5-3mPeU-T5)-57-001

Peak No.	Time	Type	Height(μV)	Area(μV-sec)	Area%
1	6.475	N	644	41216	0.319
2	9.278	N	5617	59227	0.459
3	11.558	N1	1958	12057	0.093
4	11.861	N2	2005	18375	0.142
5	12.148	N3	8473	70285	0.545
6	12.376	N4	10498	88046	0.683
7	12.818	N1	3641	39320	0.305
8	13.205	N2	24408	297032	2.306
9	14.030	N1	14346	157700	1.224
10	14.261	N2	42605	453999	3.524
11	14.538	N3	53094	952384	7.394
12	14.990	N4	87288	1624589	12.612
13	15.456	N5	68955	1178169	9.147
14	15.763	N6	832855	6573164	51.033
15	15.991	N7	70216	645671	5.012
16	16.160	N8	52613	510519	3.963
	19.465	N	745	6481	0.050
17	20.126	N1	9435	124550	0.966
18	20.331	N2	3023	25652	0.199
	24.888	N	280	1908	0.014
Total Area				12880344	99.990

EXAMPLE 6

Synthesis of decanucleotide d(A-Me_2-Ψ-U)$_5$, (dA = 2′-deoxyadenosine, dMe_2-Ψ-U = 2′-deoxy-1,3-dimethyl-Ψ-uridine).

Synthetizer configuration: see Figure 5.

Solid support: Long chain alkylamine controlled pore glass (CPG) 80-120 mesh particle size, substitution 31.2 micromole of5′-O-dimethoxytrityl-3′-O-succinyl-1,3-dimethyl-2′-deoxy Ψ-uridine per gram of CPG.

Synthetic method: H-phosphonate method of oligonucleotide synthesis.

Nucleotide(s): 5′-O-dimethoxytrityl-1,3-dimethyl-2′-deoxy-Ψ-uridine-3-O-(H)-phosphonate and 5′-O-dimethoxytrityl-2′-deoxyadenosine-3′-O-(H)-phosphonate (both compounds as triethylammonium salts).

Condensing agent: Trimethylacetyl chloride (pivaloyl chloride, PVCL).

Solvents & reagents: Acetonitrile (WGIII), 1,2-dichloroethane (WGI), 50% pyridine in acetonitrile (WGII), 2.5% dichloroacetic acid in 1,2-dichloroethane (DBLC), 2% iodine solution in pyridine(98)-water(2) (MDI).

Reaction column: 6.5 mm ID glass column with adjustable length of the bed.

Scale (loading): 0.5 micromole (= 16 mg of modified CPG).

Nucl. concentration: 0.1 mol/l solution of fully protected nucleotides in WGII (15 fold molar excess per working cycle).

Condensing agent conc.: 0.5 mol/l solution of PVCL in WGII (Ratio cond.agent:nucleotide = 5:1).

The synthesis involved the alternating delivery of nucleotide and condensing agent in Nx segments into the reaction column (as in Example 3). All steps were performed in the same manner as described in Example 3. The 2'-deoxyadenosine solution bottle was connected to the N1P position, while the 1,3-dimethyl-Ψ-2'-deoxyuridine bottle was connected to the N5P position. Repetitive yield: 94% (determined spectrophotometrically at 504 nm after each cycle as dimethoxytrityl cation). Yield: 0.05 μmole (10%) of chromatographically (HPLC) homogeneous decamer (see Table 5 and Figure 11 for results).

TABLE 5.

Data: d(A-1,3dmPU)5-54-001

Peak No.	Time	Type	Height(μV)	Area(μV-sec)	Area%
	6.163	N	130	1039	0.019
1	8.450	N	2280	24454	0.448
	10.668	N	667	5179	0.094
2	11.426	N	2441	22619	0.414
3	12.196	N	2063	20986	0.384
4	13.150	N	1023	13162	0.241
5	15.008	N	1067	10710	0.196
6	15.885	N	2870	35346	0.647
7	16.558	N	1356	15349	0.281
	16.926	N	838	7865	0.144
8	17.838	N	1061	10925	0.200
9	18.240	N	4549	58505	1.071
10	18.781	N	3758	38520	0.705
	19.258	N	908	7282	0.133
11	19.703	N1	11841	178177	3.264
12	19.948	N2	5635	108283	1.983
13	20.461	N3	8641	126898	2.325
14	20.826	N4	11004	169532	3.106
15	21.051	N5	36841	404763	7.416
16	21.281	N6	15296	189836	3.478
17	21.765	N7	50447	747162	13.689
18	22.121	N8	252297	2419680	44.333
19	22.530	N9	17382	233518	4.278
20	22.921	N10	16302	239951	4.396
21	23.218	N11	14709	225119	4.124
22	23.710	N1	4356	38128	0.698
23	24.063	N2	2771	32510	0.595
24	24.333	N3	3245	42866	0.785
25	25.881	N	1377	29524	0.540
Total Area				5457888	99.987

What is claimed is:

1. An apparatus for synthesizing an oligomer from preselected reactants which comprises:

(a) multiple reservoirs, each reservoir comprising an input means and an output means, each for holding a different one of the reactants;

(b) multiple reservoirs, each reservoir comprising an input means and an output means, each for holding a different solvent;

(c) a reaction chamber, comprising a first open end and a second open end, for holding a solid support material;

(d) a gas supply means, connected to the input means of the reservoirs, for controlling the supply of gas within the apparatus so as to maintain a constant pressure within the apparatus;

(e) a delivery valve means, comprising an input means and an output means, wherein the input means is connected to the output means of each reservoir and the output means is connected to the first open end of the reaction chamber, for selectively delivering each reactant or solvent from the output means of each reservoir to the first open end of the reaction chamber;

(f) a directional flow valve means, comprising a first port and a second port, wherein the first port is connected to the second open end of the reaction chamber, for controlling the direction of flow of each reactant and solvent;

(g) a liquid flow control means, connected to the directional flow valve means, for controlling th flow of each reactant or solvent into the liquid flow control means by negative pressure and the flow of each excess reactant or solvent out of the liquid flow control means by positive pressure;

(h) a selection valve means, comprising an input means and an output means, wherein the input means is connected to the second port of the directional flow valve means, for controlling the disposition of each excess reactant or solvent flowing from the second port of the directional flow valve means; and

(i) a control means for controlling the selection and delivery of each reactant or solvent by the delivery valve means, the direction of flow of each reactant or solvent by the directional flow valve means, the flow of each reactant or solvent by the liquid flow control means, and the disposition of each excess reactant or solvent by the selection valve means, in response to selected conditions.

2. An apparatus of claim 1 which further comprises a recycling reservoir comprising an input means and an output means, wherein the input means is connected to the output means of the selection valve means and the output means is connected to the input means of the delivery valve means, for holding each excess reactant or solvent before delivery by the delivery valve means.

3. An apparatus of claim 1 which further comprises:

(a) a recycling reservoir, comprising an input/output means, for holding each excess reactant or solvent;

(b) a recycling valve means, comprising a first port, a second port, a third port, and a fourth port, wherein the first port is connected to the output means of the selection valve means, the second port is connected to the input/output means of the recycling reservoir, the third port is connected to the first open end of the reaction chamber, and the fourth port is connected to the output means of the delivery valve means, for recycling excess reactant or solvent back through the reaction chamber; and

(c) a control means, for controlling the recycling of excess reactant or solvent by the recycling valve means.

4. An apparatus of claim 3, which further comprises:

(a) a bidirectional valve means, comprising a first port, a second port, a third port, and a fourth port, wherein the first port is connected to the third port of the recycling valve means, the second port is connected to the first opening end of the reaction chamber, the third port is connected to the second opening end of the reaction chamber, and the fourth port is connected to the first port of the directional flow valve; and

(b) a control means for controlling the bidirectional valve means.

5. An apparatus of claim 1 which further comprises:

(a) additional reservoirs, each reservoir comprising an input means and an output means, each for holding an additional reactant;

(b) additional reservoirs, each reservoir comprising an input means and an output means, each for holding an additional solvent;

(c) a modifying valve means, comprising an input means and an output means, wherein the input means is connected to the output means of each additional reservoir and the output means is connected to input means of the delivery valve means, for selectively delivering each additional reactant or solvent to the delivery valve means; and

(d) a control means, for controlling the selection and delivery of each additional reactant or solvent to the delivery valve means by th modifying valve means.

6. An apparatus of claim 2 which further comprises:

(a) additional reservoirs, each reservoir comprising an input means and an output means, each for holding an additional reactant;

(b) additional reservoirs, each reservoir comprising an input means and an output means, each for holding an additional solvent;

(c) a modifying valve means, comprising an input means and an output means, wherein the input means is connected to the output means of each

additional reservoir and the output means is connected to input means of the delivery valve means, for selectively delivering each additional reactant or solvent to the delivery valve means; and

(d) a control means, for controlling the selection and delivery of each additional reactant or solvent to the delivery valve means by the modifying valve means.

7. An apparatus of claim 3 which further comprises:

(a) additional reservoirs, each reservoir comprising an input means and an output means, each for holding an additional reactant;

(b) additional reservoirs, each reservoir comprising an input means and an output means, each for holding an additional solvent;

(c) a modifying valve means, comprising an input means and an output means, wherein the input means is connected to the output means of each additional reservoir and the output means is connected to input means of the delivery valve means, for selectively delivering each additional reactant or solvent to the delivery valve means; and

(d) a control means, for controlling the selection and delivery of each additional reactant or solvent to the delivery valve means by the modifying valve means.

8. An apparatus of claim 4 which further comprises

(a) additional reservoirs, each reservoir comprising an input means and an output means, each for holding an additional reactant;

(b) additional reservoirs, each reservoir comprising an input means and an output means, each for holding an additional solvent;

(c) a modifying valve means, comprising an input means and an output means, wherein the input means is connected to the output means of each additional reservoir and the output means is connected to input means of the delivery valve means, for selectively delivering each additional reactant or solvent to the delivery valve means; and

(d) a control means, for controlling the selection and delivery of each additional reactant or solvent to the delivery valve means by th modifying valve means.

9. An apparatus of claim 1 wherein the oligomer is an oligonucleotide.

10. An apparatus of claim 1 wherein the oligomer is an oligopeptide.

11. An apparatus of claim 1 wherein the liquid flow control means is a syringe.

12. An apparatus of claim 1 wherein the output means of the selection valve means is connected to a waste reservoir.

13. An apparatus of claim 1 wherein the output means of the selection valve means is connected to a fraction collector.

14. An apparatus of claim 1 wherein the output means of the selection valve means is connected to a waste reservoir and a fraction collector.

15. An apparatus of claim 1 wherein the control means is a computer.

16. A method for synthesizing an oligomer in a reaction area from preselected reactants which comprises:

(a) displacing one of the preselected reactants by negative pressure into the reaction area, under suitable conditions for permitting reaction;

(b) removing excess reactant from the reaction area by negative pressure;

(c) repeating steps (a) and (b) a selected number of times until reaction has occurred;

(d) displacing a preselected solvent by negativ pressure into the reaction area, under suitable conditions for permitting reaction;

(e) removing solvent from the reaction area by negative pressure;

(f) repeating steps (d) and (e) a suitable number of times until the reaction area is suitably washed;

(g) repeating steps (a)-(c) with the next preselected reactant;

(h) repeating steps (d)-(f) with the next selected solvent; and

(i) repeating steps (g) and (h) until the oligomer desired has been synthesized.

FIGURE 1

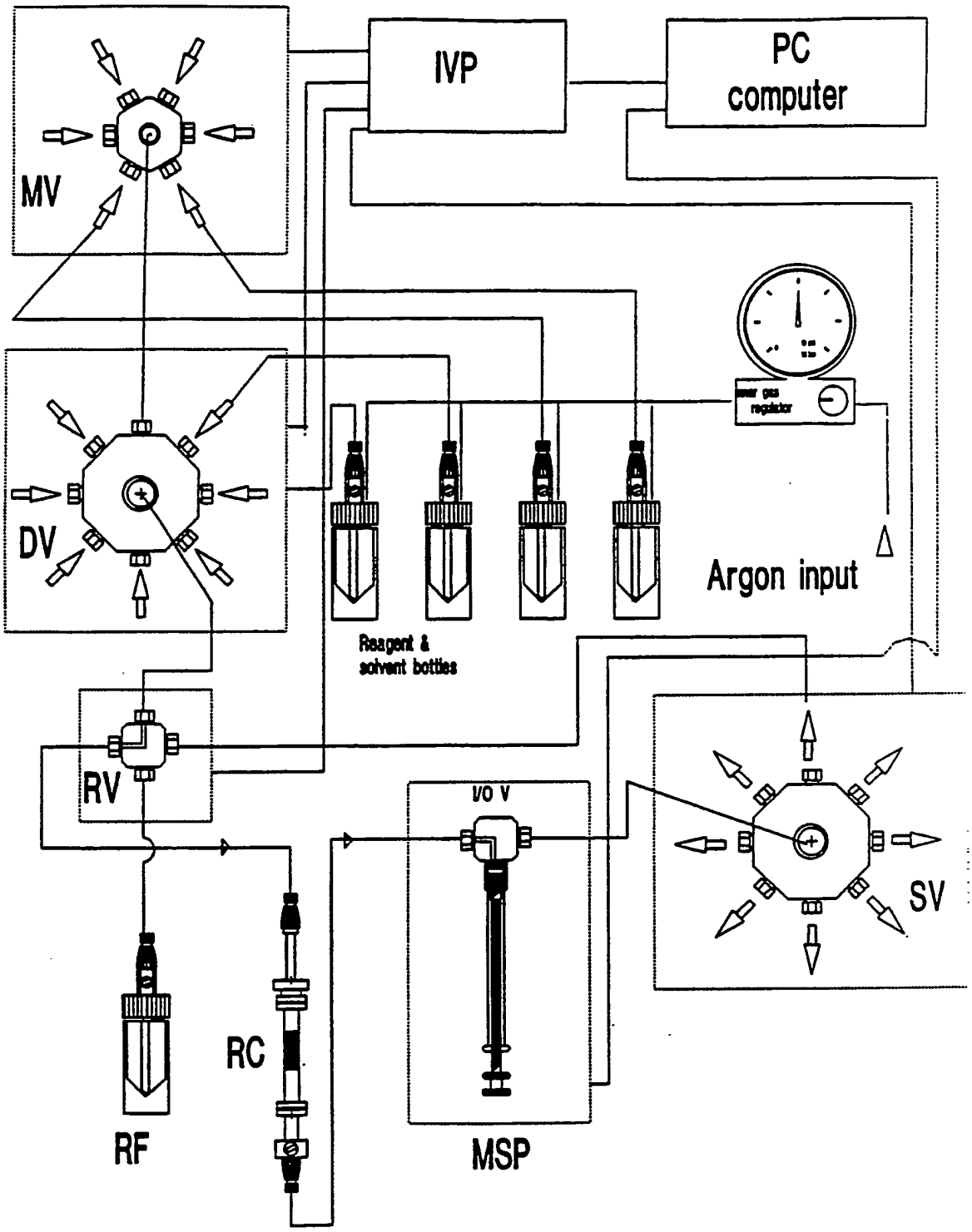

IVP
PC
computer
MV
DV
RV
SV
I/O V
MSP
RC
RF
Argon input
Reagent &
solvent bottles

FIGURE 2

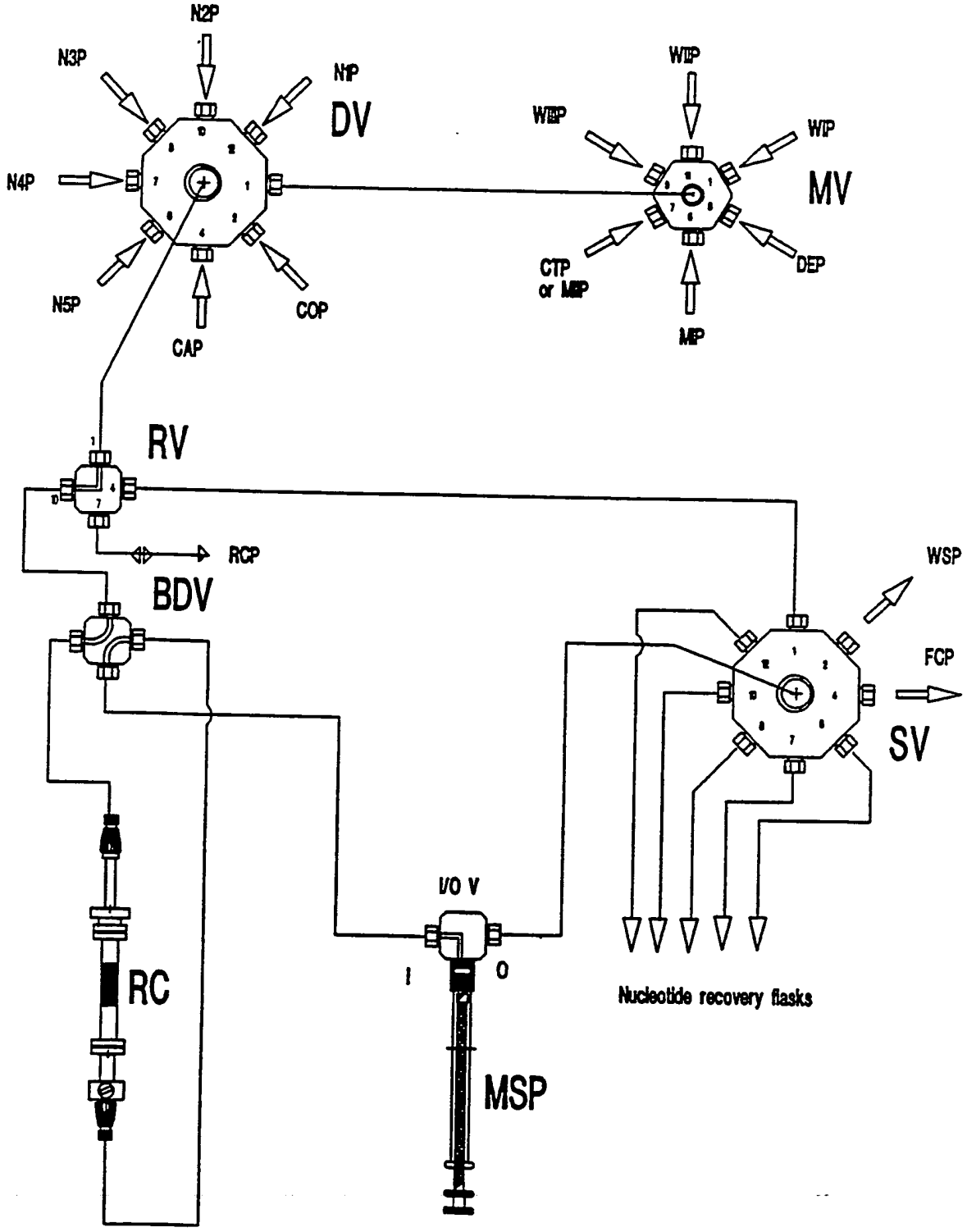

N2P
N3P
N1P
DV
N4P
N5P
CAP
COP
WIP
WIP
WIP
MV
CTP
or MIP
DEP
MIP
RV
RCP
BDV
WSP
FCP
SV
I/O V
I
O
RC
MSP
Nucleotide recovery flasks

FIGURE 3

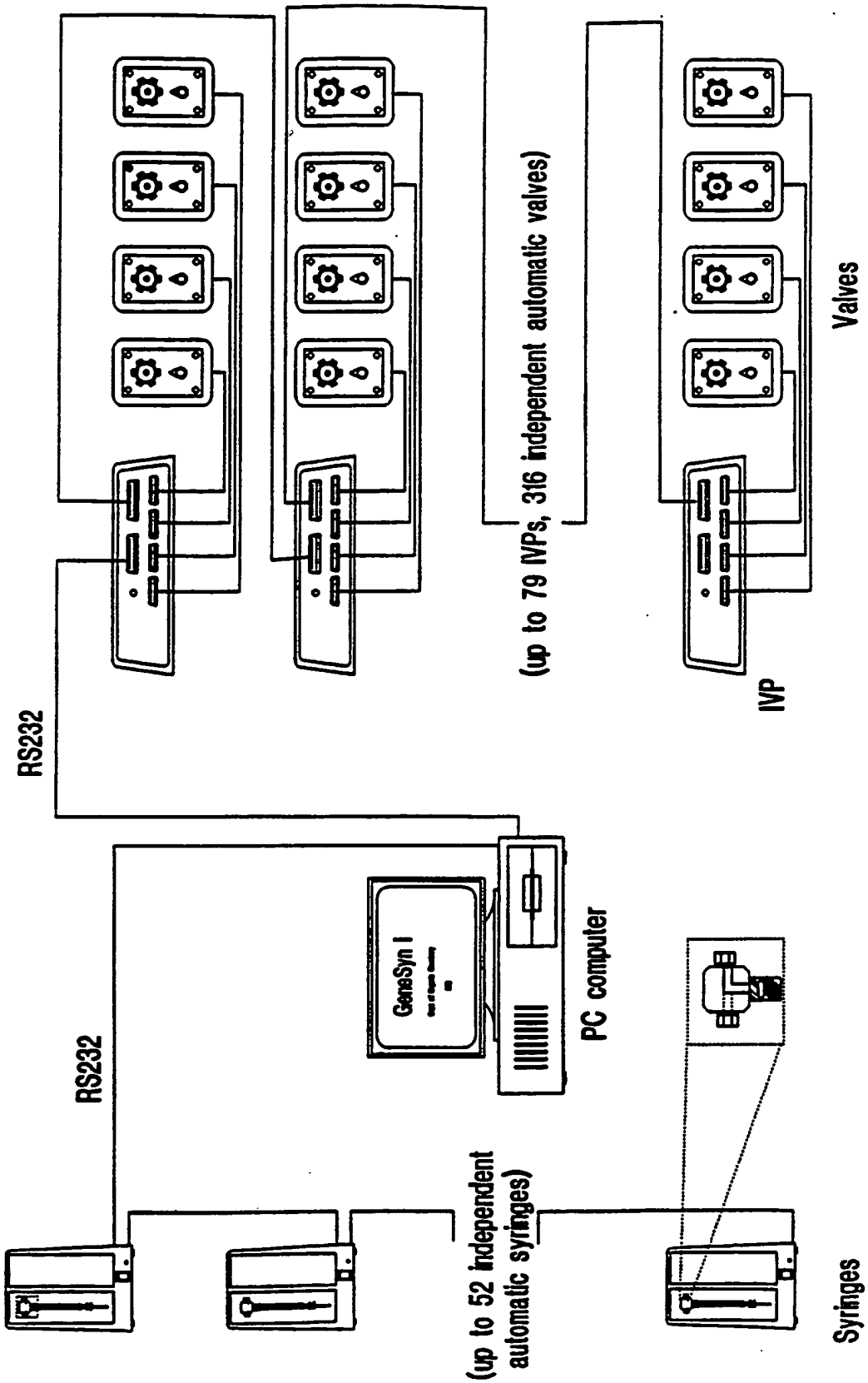

RS232
(up to 79 IVPs, 316 independent automatic valves)
IVP
Valves
RS232
GenSyn I
PC computer
(up to 52 independent automatic syringes)
Syringes

FIGURE 4

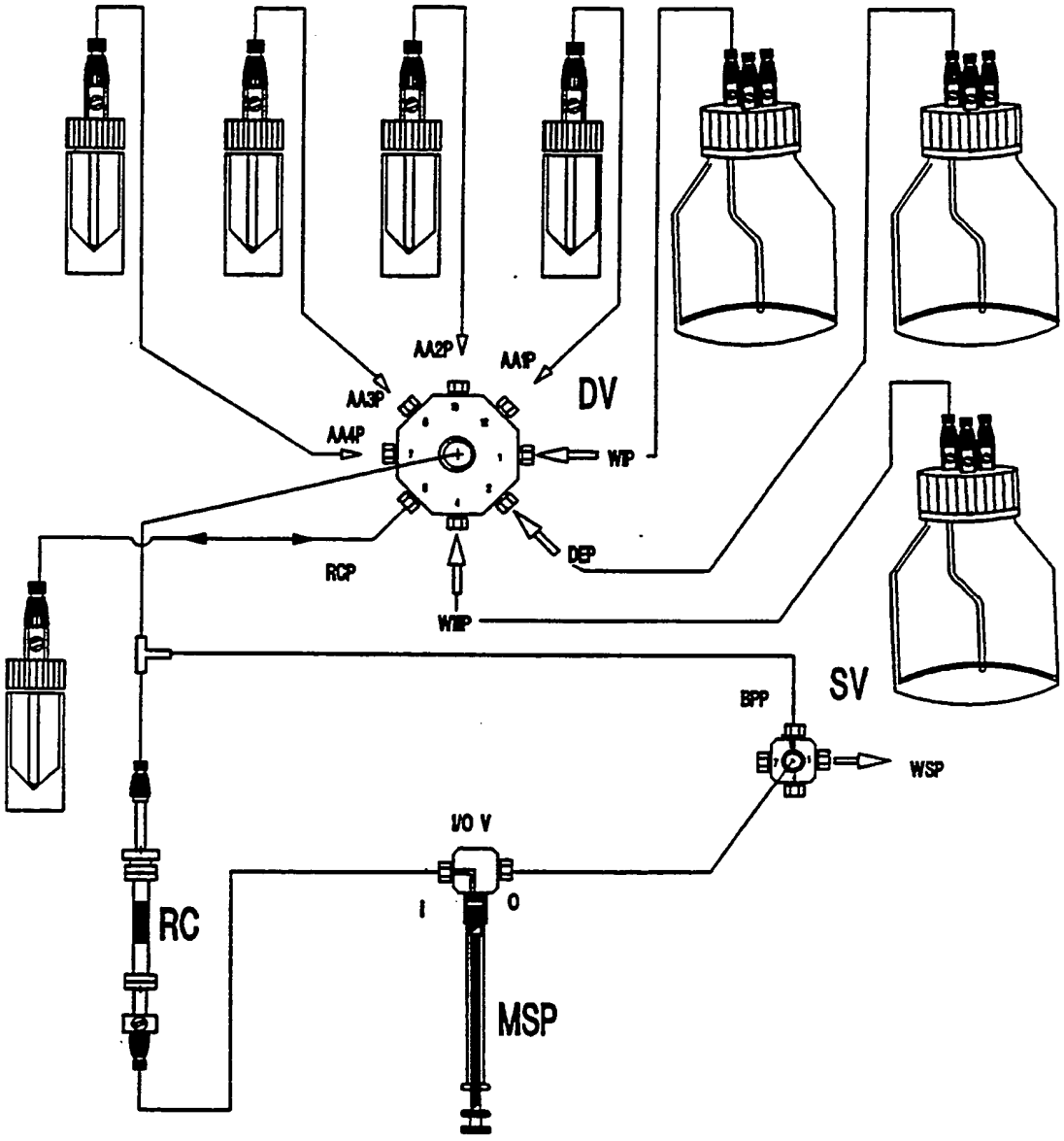


FIGURE 5

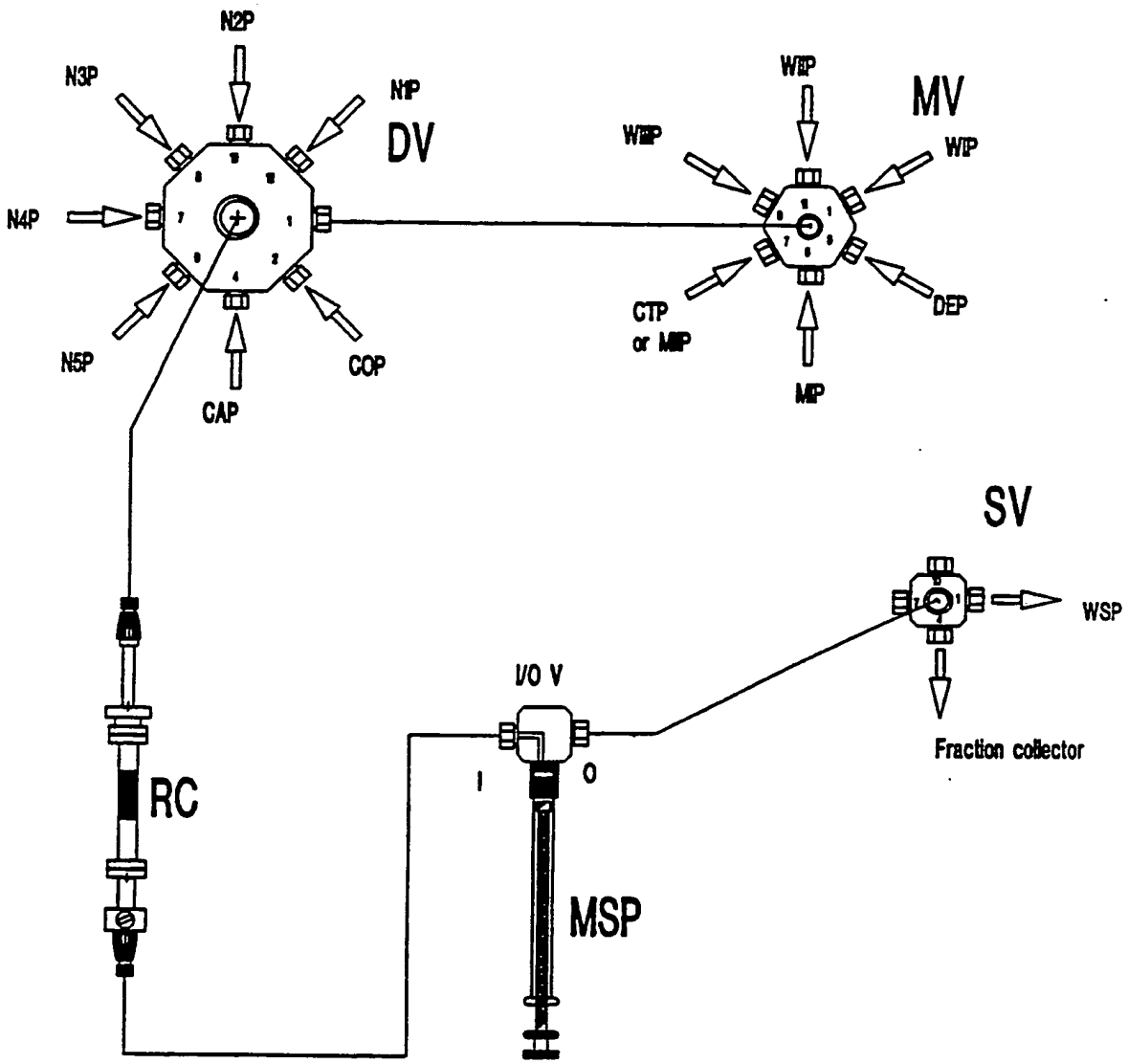

N2P
N3P
N1P
DV
N4P
N5P
CAP
COP
WIP
MV
WIP
WIP
CTP
or MIP
DEP
MIP
SV
WSP
Fraction collector
I/O V
I
O
RC
MSP

FIGURE 6

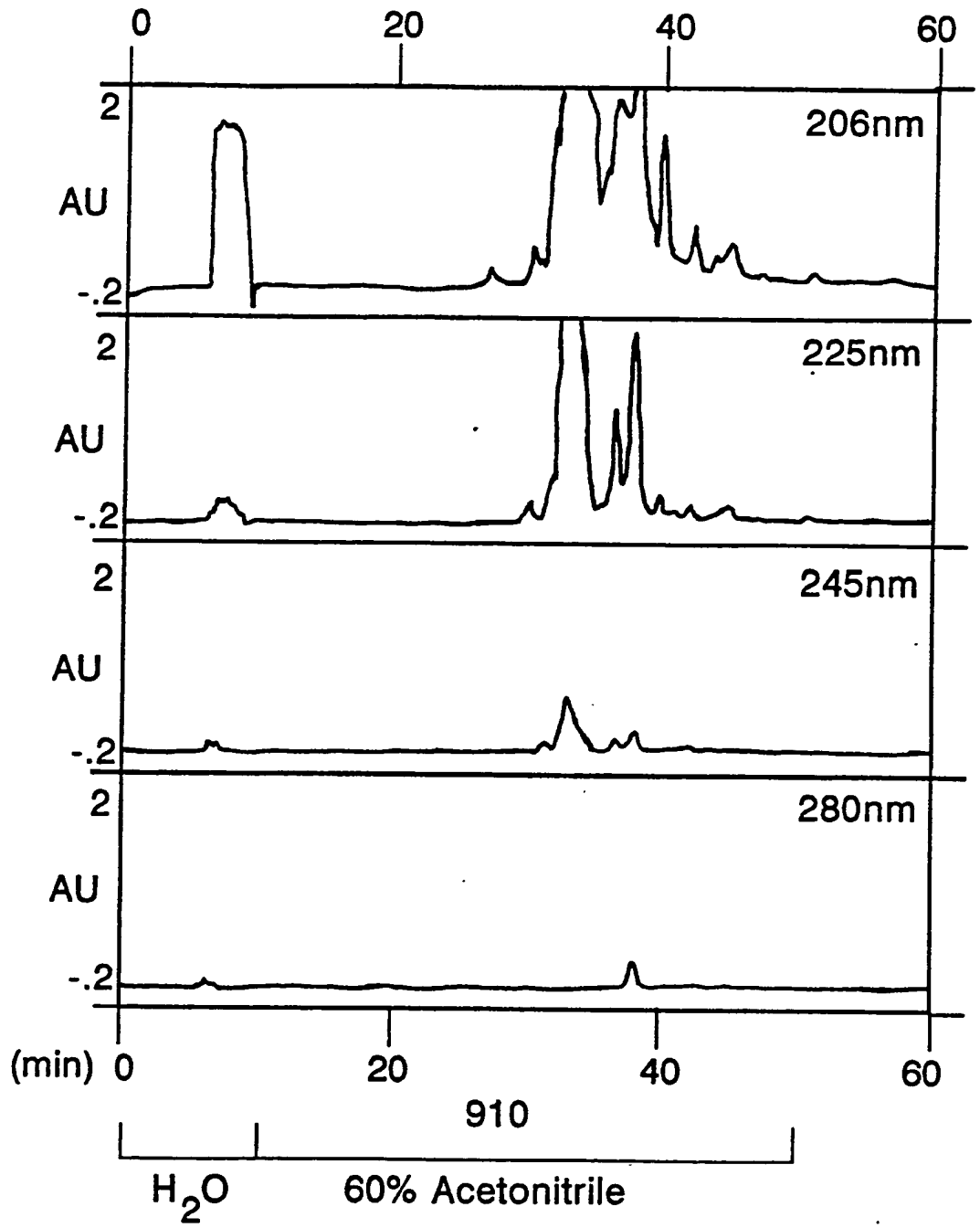

0
20
40
60
2
AU
-.2
206nm
225nm
245nm
280nm
(min) 0
20
40
60
910
H_2O
60% Acetonitrile

FIGURE 7

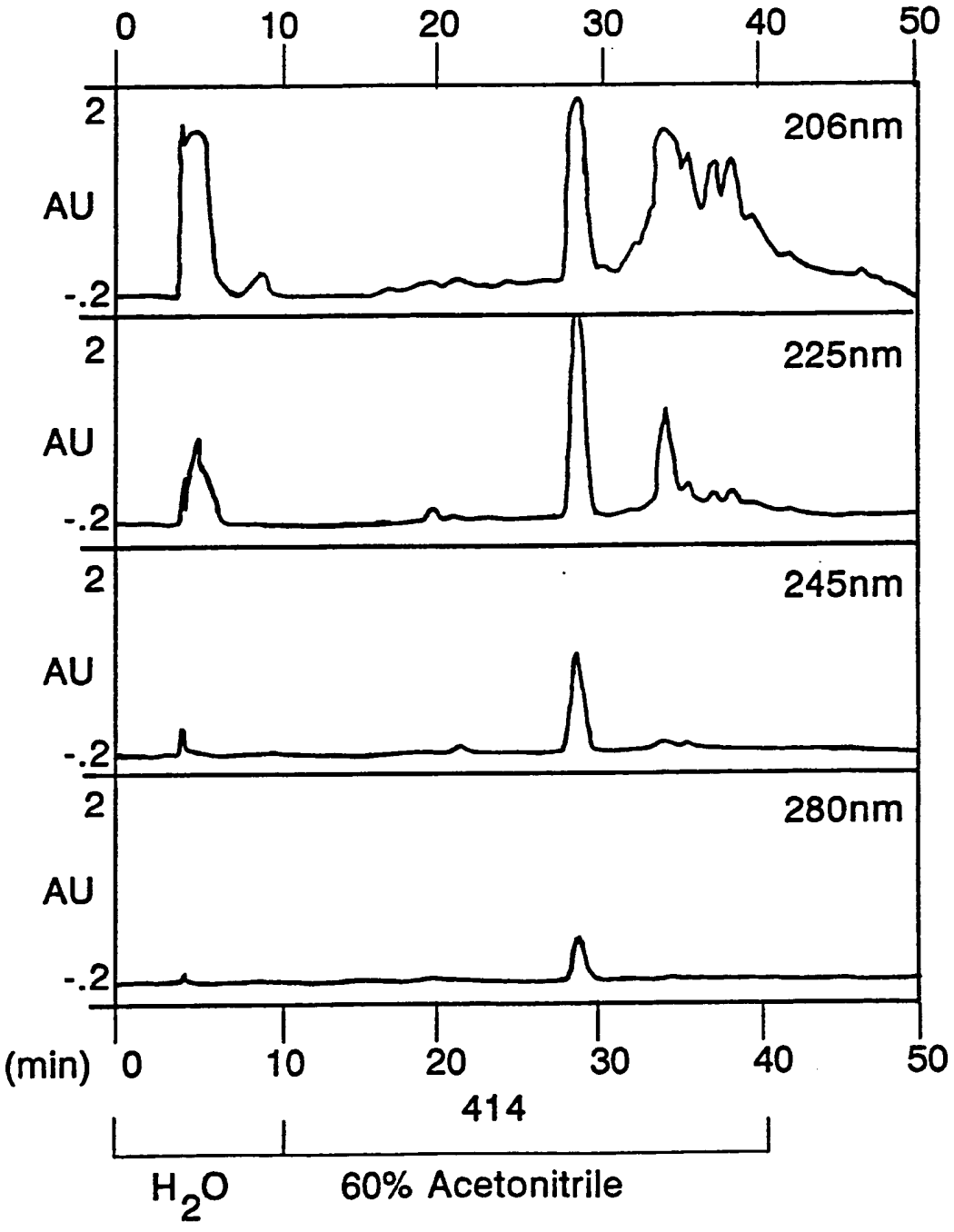

0
10
20
30
40
50
2
AU
-.2
206nm
225nm
245nm
280nm
(min)
414
H_2O
60% Acetonitrile

FIGURE 8

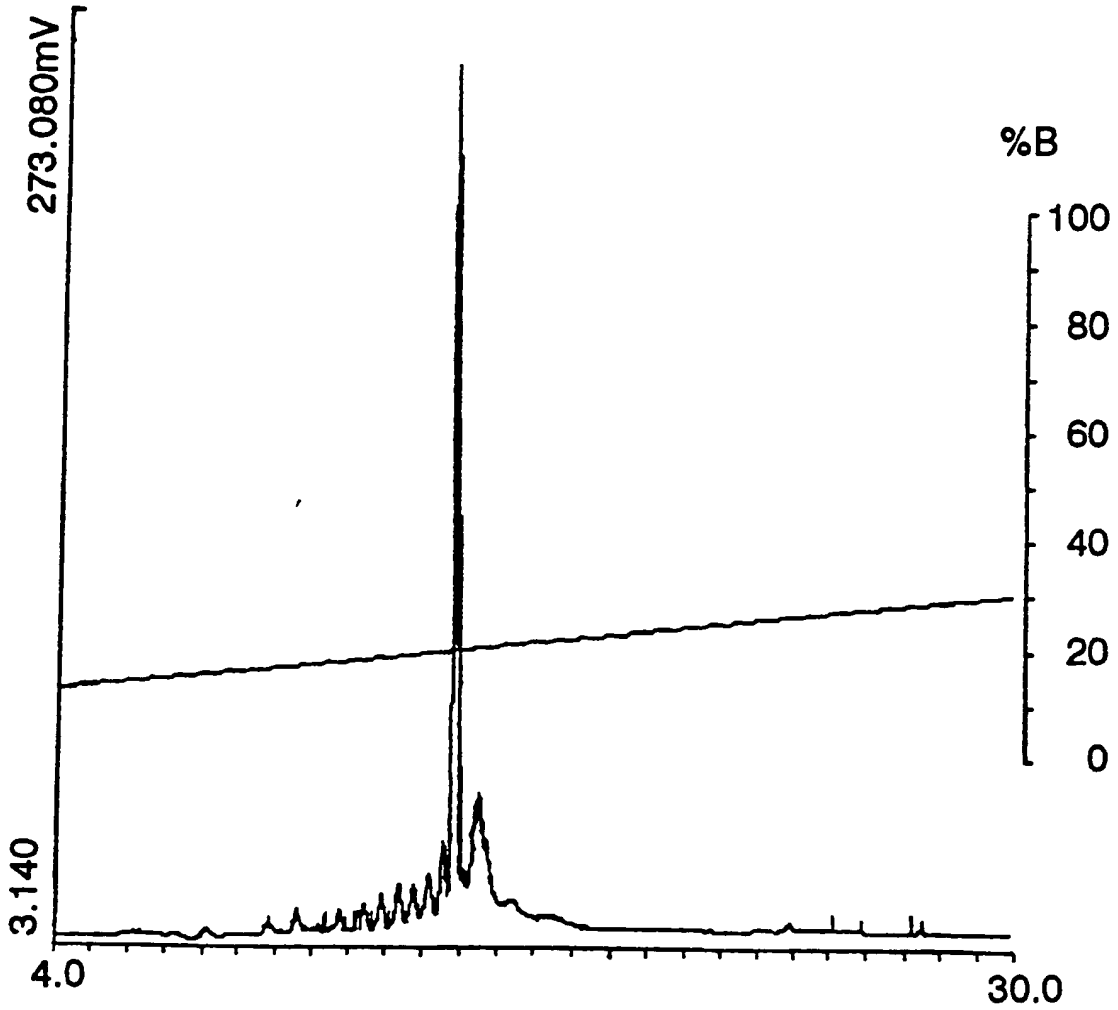


FIGURE 9

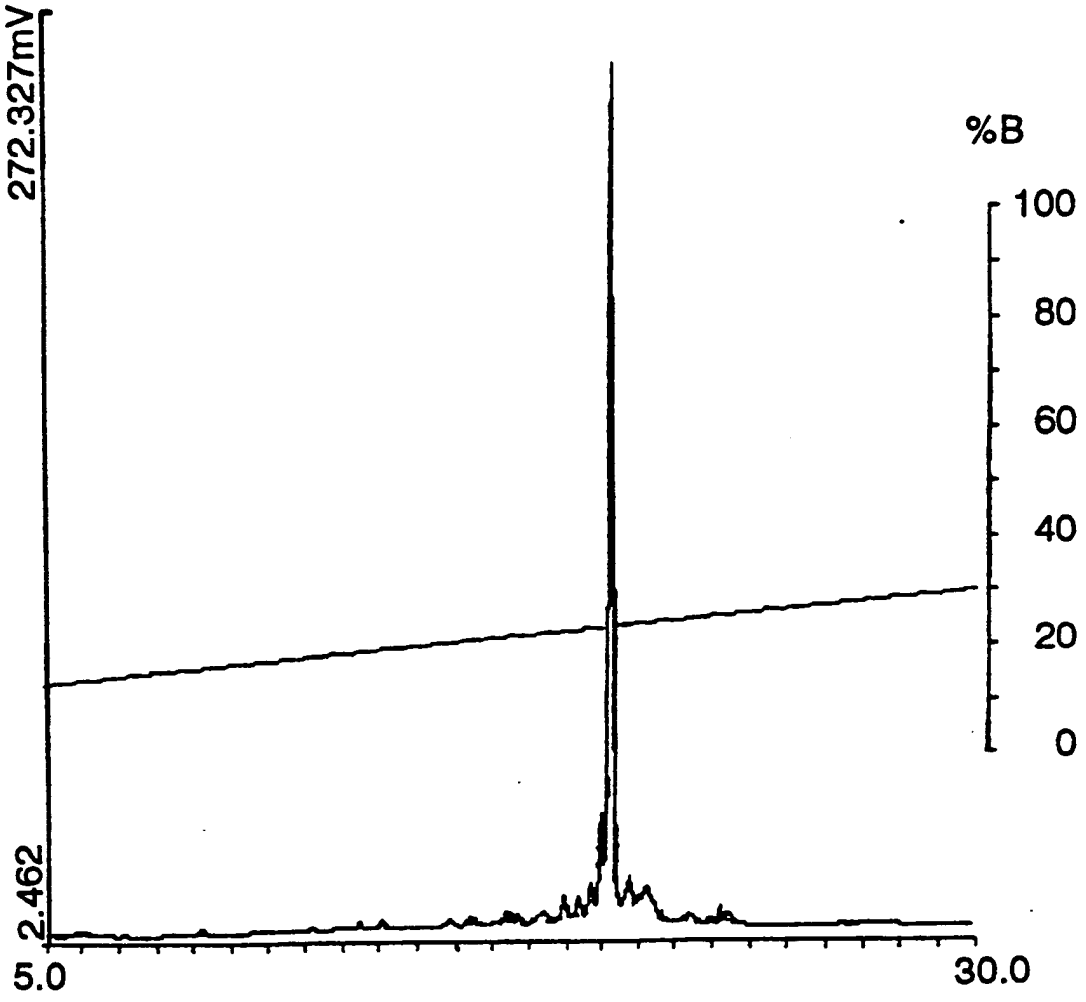


FIGURE 10

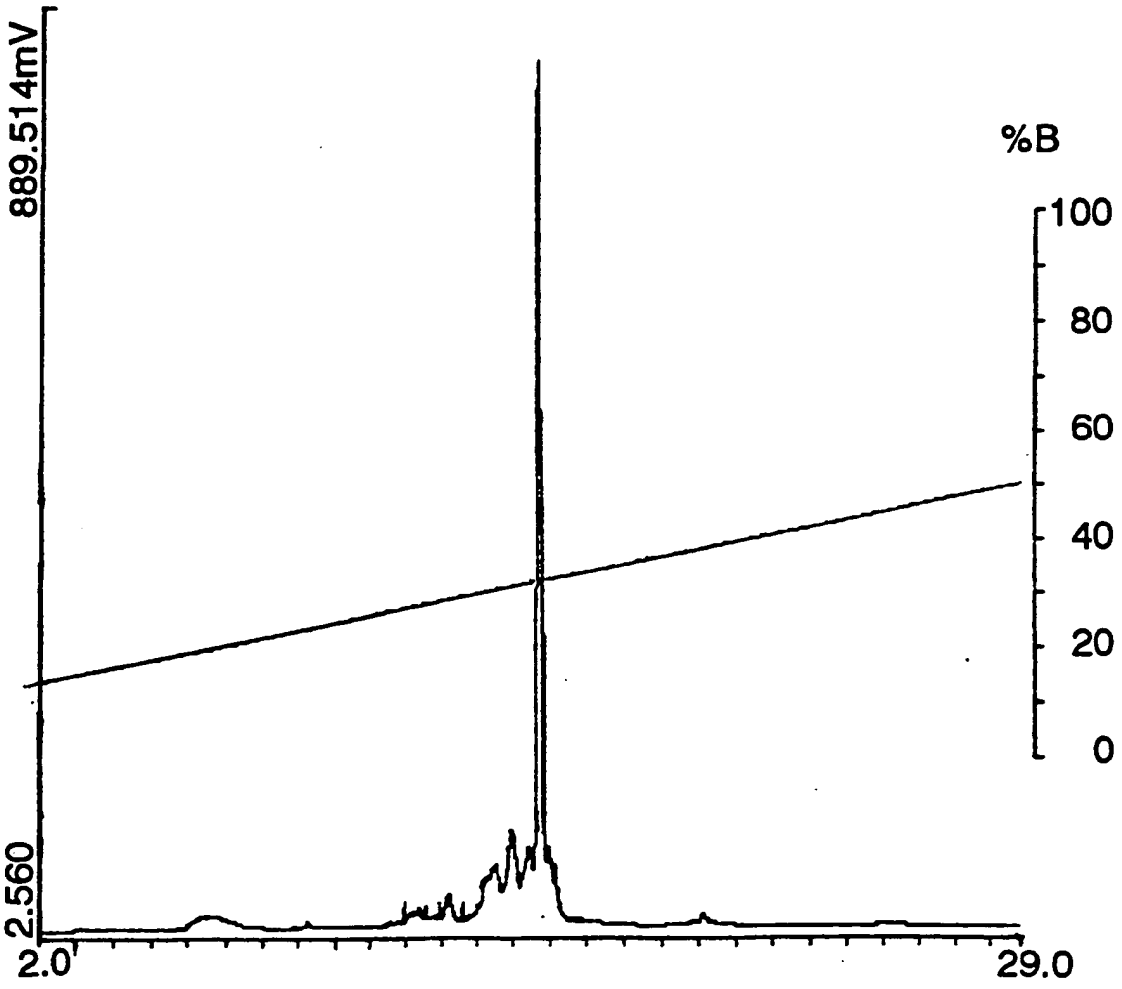
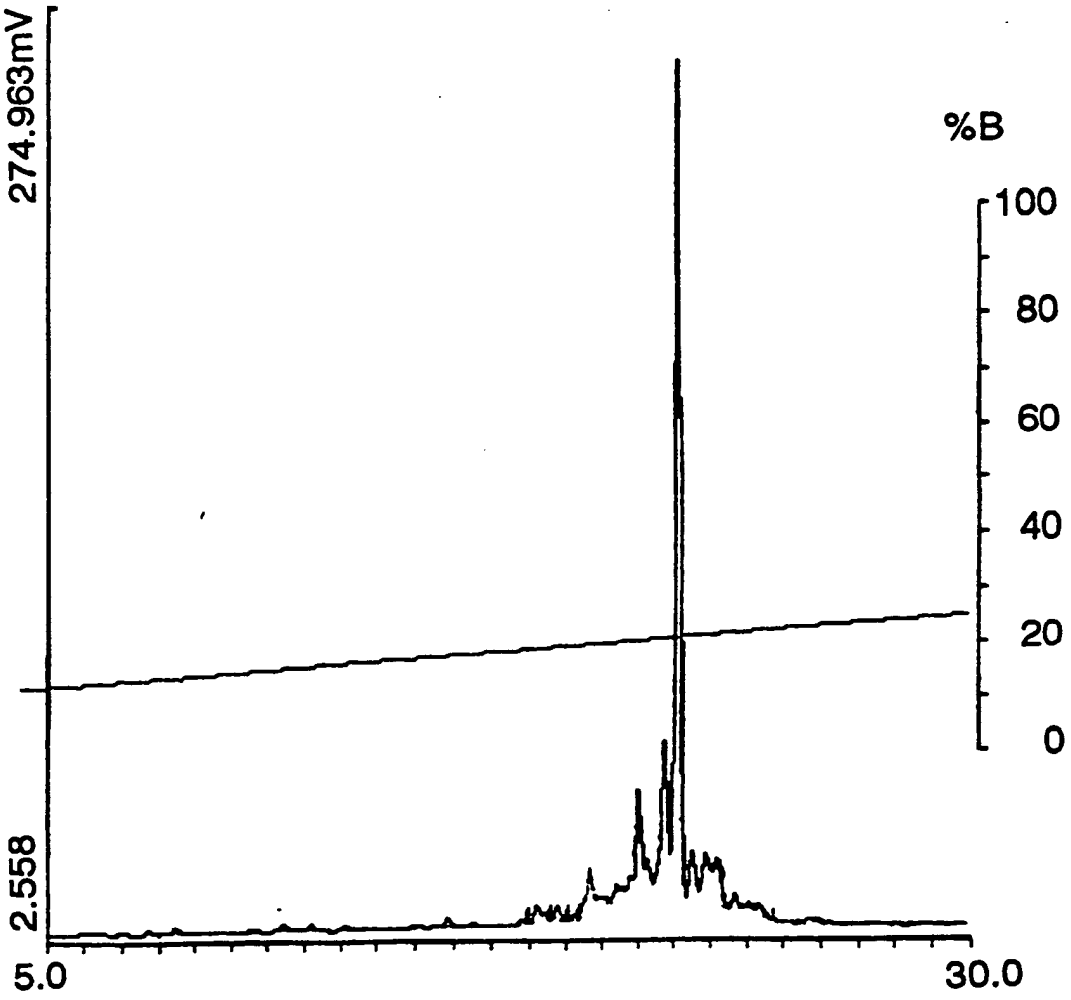


FIGURE 11



INTERNATIONAL SEARCH REPORT

Intel. ional application No.
PCT/US92/05310

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :G05D 7/00
US CL :422/81, 111, 115, 116; 935/88
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 422/81, 110, 111, 114, 115, 116; 935/88; 435/287, 288, 289; 525/54.11

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,362,699 (Verlander et al.) 07 December 1982, col. 1, lines 12-19, 27-35, col. 6, line 15, fig. #12,24-30,64,66,69,71,73,75a-d,94,98,104,and 118.	1-15
Y	US, A, 4,701,304 (Horn et al.) 20 October 1987, col. 4, lines 59-66, col. 6, lines 51-68, col. 7, lines 1-45, col. 9, line 30.	1-15
Y	US, A, 4,671,941 (Niina et al.) 09 June 1987, col. 3, lines 3-5, 9-10.	3-4 and 7-8
Y	US, A, 4,668,476 (Bridgham et al.) 26 May 1987, col. 8, lines 48-63, fig. 1a, "fraction collector", "waste reservoir".	13-14
Y	US, A, 4,598,049 (Zelinka et al.) 07 July 1986, col. 1, lines 6-8.	9-10
A	US, A, 4,746,490 (Saneii) 24 May 1988, entire document.	1-15
A,P	US, A, 5,112,575 (Whitehouse et al.) 12 May 1992, entire document.	1-15

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 02 September 1992	Date of mailing of the international search report 15 SEP 1992
Name and mailing address of the ISA/ Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer THERESA A. TREMBLEY Telephone No. (703) 308-3913

Form PCT/ISA/210 (second sheet)(July 1992)*